ABSTRACT

Disclosed is a method for determining a sequence of one or more nucleotides in a target polynucleotide. In the method, a target-specific primer is contacted with a polynucleotide sample under conditions effective for the primer to anneal specifically to a primer-complementary region in one or more target polynucleotides, to form one or more target-primer hybrid(s), wherein each target-specific primer contains (i) a target binding segment and (ii) a mobility-reducing moiety which does not bind the target. The hybrid(s) are reacted with a primer extension reagent in the presence of at least one labeled 3'-nucleotide terminator complementary to a selected nucleotide base-type, optionally in the presence of one or more extendable nucleoside monomers, under conditions effective to form one or more primer extension products which terminate with at least one labeled 3'-nucleotide terminator. The primer extension products are then separated electrophoretically in a sieving matrix under denaturing conditions such that smaller extension products migrate more rapidly than larger extension products, and the desired sequence information is determined.